

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :
Charlotte A. Kensil et al. :
Serial No. 07/573,268 : Art Unit: 183
Filed: August 27, 1990 : Examiner: N. Carson
For: SAPONIN ADJUVANT : Atty Docket: 0614.0690001

DECLARATION UNDER 37 C.F.R. § 1.131

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

We, Charlotte A. Kensil and Dante J. Marciani, do hereby
declare as follows:

1. We are the inventors of the subject matter claimed in
the above-referenced patent application.

2. Prior to December 16, 1986, we had isolated substan-
tially pure saponins as claimed and described in the subject
application.

3. The following experiments, evidenced by the accom-
panying exhibits, were performed prior to December 16, 1986, in
this country.

4. Exhibits A-L are laboratory notebook pages from the
laboratory notebook of Charlotte Kensil. These pages demonstrate
the extraction and isolation of substantially pure saponin as
claimed in the above-referenced application.

5. Exhibit A sets forth the preliminary preparation of
bark extract. This work essentially corresponds with Example 1

of the patent application. The process resulted in an extract denoted as "47-C".

6. As shown in Exhibit B, this extract, 47-C, was subjected to three extractions with methanol. Methanol extracts, 69-A and 69-B, were pooled and evaporated on a rotoevaporator to dryness. The precipitant was redissolved in 5.5mls of methanol and filtered through a 0.2 μ nylon 66 filter mesh to remove residual undissolved material. As seen on page 2 of the exhibit, two fractions were recovered: 75-B which corresponded to the precipitant on the membrane; and 75-A which corresponded to the filtered material.

7. Exhibit C sets forth reverse-phase high pressure liquid chromatography, (RP-HPLC) of dialyzed, methanol-soluble bark extract. The fraction obtained from the methanol extraction of 47-C, (75-A) was dried, redissolved, and subjected to HPLC.

8. Exhibits D and E represent results from HPLC separation. As seen, distinct peaks were visualized by RP-HPLC. That is, the individual components of bark extract were separable by RP-HPLC. Exhibits D and E depict the refractive index profile of the peaks in order of increasing retention times from RP-HPLC.

9. Reverse-phase HPLC is utilized to further purify the bark extracts. The fractions were dissolved in the appropriate solvent and loaded on Vydac C₄. A methanol gradient was used to elute the fractions.

10. The purity of individual fractions was demonstrated by either reverse-phase or normal-phase thin layer chromatography. Examples demonstrating purity of the fractions can be seen in

Exhibits F and J. As shown, the individual fractions correspond to single bands on reverse phase-silica gels.

11. Exhibit G shows both a refractive index profile and pictures of single bands on reverse phase-silica gels.

12. Exhibits H, I and K show refractive index profiles of methanol-solubilized bark extract on reverse-phase HPLC. In fact, Exhibit K corresponds to Figure 1 of the patent application. In Figure 1 of the patent application, however, the letter designations were changed to "QA" designations. The profile of the exhibit can be easily matched with that of Figure 1.

13. Exhibit L demonstrates that the refractive index peaks of the bark extract correspond to carbohydrate peaks. This information was included in the patent application as Figure 2.

14. Each of the dates deleted from Exhibits A-L is prior to December 16, 1986.

15. Therefore, prior to December 16, 1986, we had isolated substantially pure saponins and demonstrated that the purified saponins were characterized by carbohydrate content.

16. I further declare that all statements made are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false

statements may jeopardize the validity of the application or document or any registration resulting therefrom.

11/2/90
Date

Charlotte A. Kensil
Charlotte A. Kensil

11/2/90
Date

Dante J. Marciani
Dante J. Marciani

P105-10.WP5